ORIGINAL ARTICLE

Journal of Cachexia, Sarcopenia and Muscle 2018; **9**: 269–278
Published online 19 January 2018 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12268

Pitfalls in the measurement of muscle mass: a need for a reference standard

Fanny Buckinx[1]*, Francesco Landi[2], Matteo Cesari[3,4], Roger A. Fielding[5], Marjolein Visser[6,7], Klaus Engelke[8], Stefania Maggi[9], Elaine Dennison[10], Nasser M. Al-Daghri[11], Sophie Allepaerts[12], Jurgen Bauer[13], Ivan Bautmans[14], Maria Luisa Brandi[15], Olivier Bruyère[1], Tommy Cederholm[16], Francesca Cerreta[16], Antonio Cherubini[17], Cyrus Cooper[10,18], Alphonso Cruz-Jentoft[19], Eugene McCloskey[20,21], Bess Dawson-Hughes[22], Jean-Marc Kaufman[23], Andrea Laslop[24], Jean Petermans[12], Jean-Yves Reginster[1], René Rizzoli[25], Sian Robinson[10,26], Yves Rolland[27], Ricardo Rueda[28], Bruno Vellas[27] & John A. Kanis[29]

[1]*Department of Public Health, Epidemiology and Health Economics, University of Liège, Liège, Belgium;* [2]*Department of Geriatrics, Neurosciences and Orthopedics, Catholic University of the Sacred Heart Rome, Milan, Italy;* [3]*Gérontopôle, University Hospital of Toulouse, Toulouse, France;* [4]*INSERM UMR1027, University of Toulouse III Paul Sabatier, Toulouse, France;* [5]*Nutrition, Exercise Physiology and Sarcopenia Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA02111, USA;* [6]*Department of Health Sciences, VU University Amsterdam, Amsterdam, Netherlands;* [7]*Department of Nutrition and Dietetics, Internal Medicine, VU University Medical Center, Amsterdam, Netherlands;* [8]*Institute of Medical Physics, University of Erlangen, Erlangen, Germany;* [9]*National Research Council, Neuroscience Institute, Aging Branch, Padova, Italy;* [10]*MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, England, UK;* [11]*Prince Mutaib Chair for Biomarkers of Osteoporosis, Biochemistry Department, College of Science, King Saud University, Riyadh11451, Saudi Arabia;* [12]*Department of Geriatrics, CHU-Liège, Liège, Belgium;* [13]*Department of Geriatric Medicine, Klinikum, Carl von Ossietzky University, Oldenburg, Germany;* [14]*Gerontology and Frailty in Ageing Research Department, Vrije Universiteit Brussel (VUB), Brussels, Belgium;* [15]*Department of Surgery and Translational Medicine, University of Florence, viale Pieraccini 6*59139, Florence, Italy; [16]*Human Medicines Research and Development Support Division, Scientific Advice, London, UK;* [17]*Geriatrics and Geriatric Emergency Care, IRCCS-INRCA, Ancona, Italy;* [18]*NIHR Musculoskeletal Biomedical Research Unit, University of Oxford, Oxford, UK;* [19]*Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria (Irycis), Madrid, Spain;* [20]*Centre for Metabolic Bone Diseases, University of Sheffield, Sheffield, UK;* [21]*MRC and Arthritis Research UK Centre for Integrated research in Musculoskeletal Ageing (CIMA), London, UK;* [22]*Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA;* [23]*Department of Endocrinology and Unit for Osteoporosis and Metabolic Bone Diseases, Ghent University Hospital, Ghent, Belgium;* [24]*Scientific Office, Austrian Agency for Health and Food Safety, Vienna, Austria;* [25]*Service of Bone Diseases, Department of Internal Medicine Specialties, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland;* [26]*National Institute for Health Research Southampton Biomedical Research Centre, University of Southampton and University Hospital, Southampton NHS Foundation Trust, Southampton, UK;* [27]*Gérontopôle de Toulouse, Institut du Vieillissement, Centre Hospitalo-Universitaire de Toulouse (CHU Toulouse); UMR INSERM 1027, University of Toulouse III, Toulouse, France;* [28]*Abbott Nutrition R&D, Granada, Spain;* [29]*Centre for Metabolic Bone Diseases, University of Sheffield, UK and Institute of Health and Ageing, Australian Catholic University, Melbourne, Australia*

Abstract

Background All proposed definitions of sarcopenia include the measurement of muscle mass, but the techniques and threshold values used vary. Indeed, the literature does not establish consensus on the best technique for measuring lean body mass. Thus, the objective measurement of sarcopenia is hampered by limitations intrinsic to assessment tools. The aim of this study was to review the methods to assess muscle mass and to reach consensus on the development of a reference standard.

Methods Literature reviews were performed by members of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis working group on frailty and sarcopenia. Face-to-face meetings were organized for the whole group to make amendments and discuss further recommendations.

Results A wide range of techniques can be used to assess muscle mass. Cost, availability, and ease of use can determine whether the techniques are better suited to clinical practice or are more useful for research. No one technique subserves all requirements but dual energy X-ray absorptiometry could be considered as a reference standard (but not a gold standard) for measuring muscle lean body mass.

Conclusions Based on the feasibility, accuracy, safety, and low cost, dual energy X-ray absorptiometry can be considered as the reference standard for measuring muscle mass.

Keywords Lean mass; Muscle mass; Lean body mass; Reference standard

Received: 4 May 2017; Revised: 5 September 2017; Accepted: 12 October 2017
**Correspondence to: Fanny Buckinx, M.Sc., PhD candidate, University of Liège, Department of Public Health, Epidemiology and Health Economics, CHU-Sart-Tilman, B23, Quartier Hôpital, Avenue Hippocrate, 13, 4000 Liège, Belgium. Tel.: +32 4366 49 33, Email: fanny.buckinx@ulg.ac.be*

Background

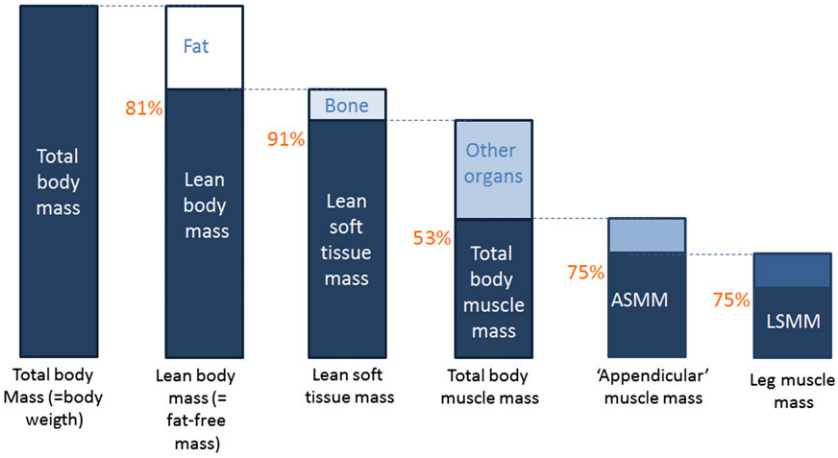
The term sarcopenia was first used by Rosenberg et al. in 1989[1] to refer to a progressive loss of skeletal muscle mass with advancing age. Baumgartner, defined sarcopenia as appendicular skeletal muscle mass (kilogram)/height^2(metre^2) being less than two standard deviations below the mean of a reference group.[2] Since then, the conceptual definition of sarcopenia has expanded to include impaired muscle strength and/or physical performance. In turning a conceptual definition to an operational definition, several have been proposed,[2–10] but no consensus has yet been reached. The multidimensional nature of sarcopenia implies that its domains should be objectively assessed.[11,12] Therefore, valid, standardized, reliable, accurate, and cost-effective tools are necessary for the identification of sarcopenia.[13,14] Currently, all the proposed definitions include the measurement of muscle mass but the techniques used to assess it vary. In recent years, four main techniques have been commonly used to estimate muscle mass: bioelectric impedance (BIA), dual energy X-ray absorptiometry (DXA), computed tomography (CT), and magnetic resonance imaging (MRI) to replace anthropometry.[15–17] In addition to these, several emerging techniques for the assessment of muscle mass are now available. Each rely on different technologies and assess different aspects of muscle mass (e.g. total body muscle mass, appendicular muscle mass, or mid-thigh muscle cross-sectional area) (Figure 1). At the organizational level, the body can be separated into chemical or anatomical distinct compartments. The 2-compartment model divides the body weight into fat mass and fat free mass or FFM.[18] Body composition techniques are based on these organizational levels. Therefore, the objective measurement of sarcopenia is hampered by limitations intrinsic to assessment tools.[11,19] From a clinical and epidemiological point of view, it is important to have a consensual technique. The use of different diagnostic methods may lead to different prevalence of sarcopenia and may therefore have significant consequences on preventive or therapeutic strategies.

Matiegka reported in 1921 what was to become a classic anthropometric approach to quantifying skeletal muscle mass.[20] Matiegka's method divided body weight into four parts: skeleton, skeletal muscle, skin plus subcutaneous adipose tissue, and the remainder. Others that followed Matiegka were limited by a lack of reference standards for skeletal muscle mass measurement until the introduction of CT by Hounsfield.[21] After this phase, CT, MRI, and DXA were being used to measure muscle mass (so a more specific muscle assessment compared with the general FFM). Subsequently, BIA equations were developed to predict muscle mass (instead of FFM). The availability of DXA systems, with modest scan cost, low radiation exposure, short scan time, and extensive information provided from a whole body scan makes this approach the most widely used in sarcopenia research at the present time.[17,22] Indeed, imaging methods such as MRI and CT are expensive methods and are not accessible to the majority of clinicians and researchers.[23] Nevertheless, the literature has not established consensus on the 'best' technique to measure muscle mass. Because of the need for consensus and standardization for both clinicians and researchers, the widely used techniques measuring muscle mass are reviewed in the succeeding text and recommendations derived therefrom.

Methods

The European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis working group on frailty and sarcopenia consists of clinical scientists and experts in the field of musculoskeletal diseases. Different members of

Figure 1 Body compartments based on reference man.



DOI: 10.1002/jcsm.12268

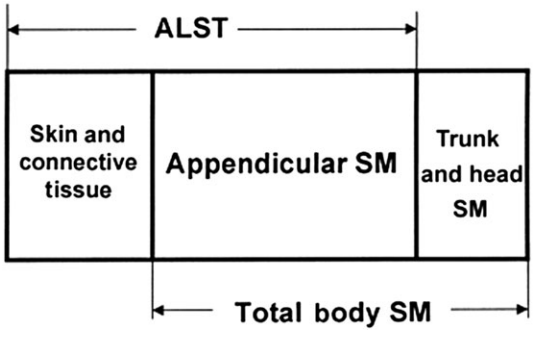
the working group were asked to prepare a literature review on the role of lean mass measurement in the assessment of sarcopenia (M.C.), the measurement of Lean Body Mass with DXA (M.V. and K.E.), with bioimpedance (S.M.), and with emerging techniques (R.F.). The topic 'how to produce reference standards for the assessment of Lean Body Mass' was also discussed (E.D.). Each member prepared a list of the most important papers based on their literature search and made a set of preliminary recommendations. For each item, a complete literature search was performed to identify new or additional randomized controlled trials and systematic reviews/meta-analysis, if any, not used in the existing guidelines. The MEDLINE (pubmed) database was searched using the name of each technique for measuring body composition as a search term, together 'with lean body mass', limiting results to 'humans', 'randomized controlled trials', 'meta-analysis', 'systematic reviews', and 'guidelines'. A similar search was adapted for the Embase database, and each item was also searched in the Cochrane Database of systematic reviews. The reference list of relevant retrieved articles was hand-searched for additional resources when member of the working group were interrogated for their knowledge on articles or congress abstract in press. A free web search was also performed and considered. Searches were performed from the year 2000 and updated until September 2016, with the additional evidence constantly provided to the working group members for selection of the best evidence according to the panel.

The subsequent step was a face-to-face meeting for the whole group to make amendments and discuss further recommendations. The plan of the manuscript was also discussed and shared conclusions were reached.

Results

First, it seems important to clarify several terms. Skeletal muscle mass is the largest component of adipose tissue–free body mass in humans.[24] Lean mass also known as lean body mass is a fat-free and bone mineral–free component that includes muscle and other components such as skin, tendons, and connective tissues (Figure 2). Appendicular lean soft tissue is the sum of lean soft tissue from both arms and legs.[26] A large proportion of total-body skeletal muscle is found in the extremities, and a large proportion of appendicular lean soft tissue is skeletal muscle (Figure 1).[25]

Figure 2 Relations between appendicular lean soft tissue (ALST) and total-body skeletal muscle (SM) mass.[25]



Measurement of lean body mass and muscle mass with imaging techniques

Dual energy X-ray absorptiometry

Dual energy X-ray absorptiometry is the most widespread technique for measuring body composition.[27] DXA uses two different energy spectra to differentiate two materials: either bone or soft tissue, which is the basis for the measurement of bone mineral density (BMD) and content or lean soft tissue mass and fat mass in locations where bone is absent. Taken together, DXA provides an estimate of three body compartments, that is, lean, bone, and fat. At bone locations, lean and soft tissue are interpolated from the surroundings. These measurements can be performed for the whole body and for several regions (e.g. trunk, arms, and legs).[28,29] The principle of using DXA for measurement of body composition is based on the notion that when a beam of X-rays is passed through a complex material, the beam is attenuated in proportion to the composition and thickness of the material. The use of two different energy spectra is the basis to separately quantify the amount of bone mineral and soft tissue or of fat and lean mass. Lean soft tissue and adipose tissue are mostly comprised by water and organic compounds, which restrict the flux of X-rays less than bone.[15,30] DXA is able to assess total body lean soft tissue mass (which includes skeletal muscle mass as well as the mass of all other organs) and appendicular lean soft tissue mass (i.e. an estimate of the muscle mass contained in the limbs, which represents about 75% total body skeletal muscle mass).[27]

Appendicular lean soft tissue mass measured by DXA is highly correlated with both MRI (r = 0.88; $P < 0.001$) and CT (r = 0.77–0.95, $P < 0.0001$) measures of skeletal muscle volume.[25,31–39] *In vivo* precision errors depend on DXA equipment, population, local versus whole body measurements, age, and degree of obesity. Recently published values for appendicular lean soft tissue mass range from below 1–3.0%. Higher errors of 4% were reported for bilateral muscle mass of the arms. Precision of DXA is high.[40] According to Hangartner, the precision error, expressed in %CV, for lean body mass was 1.2%[40].

Strengths and weakness of the DXA technique are summarized in Table 1.

Table 1 Strengths and weakness of measuring muscle mass by dual energy X-ray absorptiometry

Strengths	Weaknesses
Non-invasive with small doses of radiation (<1 μSv for whole-body scans).[41]	Projectional technique, individual muscles cannot be assessed separately.
Relatively cheap, compared with CT scan or MRI.	Not portable, which may preclude its use in large-scale epidemiological studies and studies in the home setting.
Rapid	Availability is limited in some care settings.
Allows measurement of three body compartments.	Body thickness and abnormalities in hydration status (e.g. water retention, heart, kidney, or liver failure) can affect muscle mass measure.[42]
Low precision errors	Very tall and very obese people cannot be measured.
	Cannot quantify fatty infiltration of muscle. It is a bias in the diagnosis of sarcopenia obesity.
	Does not measure skeletal muscle mass in non-limb regions of the body (e.g. trunk).
	Several devices and several software packages and software versions resulting in different results.

CT, computed tomography; MRI, magnetic resonance imaging.

Note that DXA half-body analysis in obese subjects appears to be closely comparable to whole-body analysis for fat mass, non-bone lean mass, and percent fat, though there are no data on the comparability at appendicular sites.[43]

Dual energy X-ray absorptiometry is a candidate for providing a reference technology for assessing lean mass (as a proxy of muscle mass) and body composition in research and clinical practice. There is however a need for standardization. Standardization can be approached using phantoms or humans. Existing body composition phantoms are not anthropometric and cannot be used as absolute reference standards for soft-tissue composition. Therefore, a recent International Society for Clinical Densitometry report concluded that 'No phantom has been identified to remove systematic difference in body composition when comparing in vivo results across manufacturers'. As a consequence, 'an in vivo cross-calibration study is necessary when comparing in vivo results across manufacturers'.[41]

Still for a unique standardization, the use of phantoms would be preferable because an *in vivo* cross calibration is influenced by age, gender, ethnicity, healthy versus diseased subjects, and so on.[44] Ideally, the calibration materials and equations used to derive lean mass should be standardized across manufacturers or cross-manufacturer algorithms should be developed by industry to standardize the output. It is also important to standardize the local regions of interest, such as trunk, arms, and legs, which are significantly different across manufacturers.[45,46]

Computed tomography

Computed tomography (CT) was the first method introduced that could quantify regional skeletal muscle mass with high accuracy.[17] CT determines the cross-sectional distribution of the X-ray absorption coefficient, which after normalization to the absorption of air and water is called CT value and measured in Hounsfield units (HU). CT slices of predefined width can be analysed for different tissues, using manual segmentation or automated software. For example, muscle area, or in case of the analysis of a stack of images, volume of individual muscles, or a group of muscles can be determined. By definition, the HU value of air is −1000 and of water 0. Bone, skeletal muscle, adipose tissue, and visceral organs have specific Hounsfield unit ranges, allowing for their identification in the cross-sectional images. The tissue area/volume (cm^2/cm^3) of the cross-sectional/stack of images is subsequently calculated by multiplying the number of pixels/voxels for a given tissue by the pixel area/voxel size. Muscle mass can be derived by multiplying muscle volume by 1.04 that is the assumed constant density (kg/cm^3) of adipose tissue-free skeletal muscle.[47]

Compared with DXA, CT is a 3D imaging technique that allows for quantitative assessment of individual muscles. Moreover, the muscle tissue composition can be quantified, either by separate segmentation of muscle and adipose tissue or by analysing muscle density, that is, the HU distribution within the segmented muscle.[48]

In vivo precision errors for muscle volume or mass measurements have rarely been reported, but reanalysis precision errors are low due to its high resolution (typically 50 microns or less).[49] This is important because with advanced 3D imaging, precision of muscle area and mass depend more on image segmentation than on repositioning. For reanalysis, intraclass correlation coefficients (ICC) between 0.98 and 1.00 ($P < 0.001$)[50] in quantifying both adipose tissue and muscle mass[51] were reported.

Major disadvantages of CT are limited access to the radiological departments that operate it and considerably higher cost and radiation exposure than for DXA. Despite calibration of HU to water, calibration of CT across models and scanner manufacturers is still required when comparing scans from different devices. In addition, very obese patients may not fit into the scanner and image quality will be poor. Also, the operation of a CT scanner requires highly qualified personnel. The widespread implementation of CT imaging in the field of sarcopenia has been hampered by the previously mentioned

limitations. An alternative to whole body clinical CT scanners may be the use of CT scanners dedicated and limited to peripheral investigations, which is cheaper and has lower exposure to radiation is presently better suited for small-scale research studies in which accurate measurements of muscle quantity and quality are needed.

Magnetic resonance imaging

The introduction of MRI in the 1980s expanded the initial use of CT as a means of developing 3-dimensional images of skeletal muscle, adipose tissue, and other organs. This development is usually referred to as structural or anatomic imaging.[17] The resolution is very high, and MRI is safe without any radiation exposure. With the advancement of the MRI technique, the time for reliable image acquisition has decreased significantly. In addition, most modern MRI scanners can accommodate obese subjects. Limitations in the use of MRI in clinical and research settings are largely related to the high cost, the technical expertise required for analysis, and the effect of respiratory motion on image quality for whole-body assessments. Multiple slices are required to assess the composition of the total body, including total body skeletal muscle mass.[52] Finally, the existence of multiple protocols for data acquisition impacts the standardization of this technique for the study of muscle mass.[42] Bearing all these considerations in mind, MRI is presently better suited for small-scale research studies in which accurate measurements of muscle quantity and quality are needed.

Estimation of lean body mass and muscle mass with bioimpedance analysis

Bioimpedance analysis (BIA) was pioneered in the 1950s and 1960s by Hoffer, Nyboer, and Thomasset.[53–55] Since then, BIA has become a broadly applied approach used in body composition measurements and healthcare assessment systems.[56]

BIA is based on the notion that tissues rich in water and electrolytes (i.e. skeletal muscle) are less resistant to the passage of an electrical current than lipid-rich adipose tissue (i.e. bone).[17,57] All BIA systems exploit these tissue-specific conductivity differences to quantify body-compartments. In bioimpedance measurements, the human body is divided into five inhomogeneous segments, two for the upper limbs, two for the lower limbs, and one for the trunk.[56] Many available BIA system designs range from single to multiple frequency, employ contact or gel electrodes, and measure whole-body electrical or segmental pathways.[17] All BIA systems measure impedance and/or its two components, resistance (caused by the total water across the body) and reactance (due to capacitance of cell membrane). These electrical measurements in turn can be incorporated into body composition prediction equations that are population specific.[17] Advantages and disadvantages of BIA are listed in Table 2.

Due to the large number of factors conditioning BIA reliability: instrument related factors (i.e. intra-instrumental and inter-instrumental variability, electrode quality, and electrode positioning), technician-related factors (i.e. intra-operator and inter-operator variability), subject-related factors (i.e. subject preparation such as position, overnight fast or empty bladder, body temperature, skin conductibility, age, and ethnicity), and environment-related factors (i.e. temperature), BIA does not seem to be ideal for measuring lean body mass, mainly due to the problem of the individual prediction error. A recent study showed that the reliability of BIA to assess appendicular lean mass was high, with an ICC of 0.89 (95%CI: 0.86–0.92) when performed by the same operator, and an ICC of 0.77 (95%CI: 0.72–0.82) when performed by two different operators. Nevertheless, in this study, agreement between appendicular lean mass assessed by DXA and predicted by BIA was low [ICC = 0.37 (95%CI: 0.25–0.48)].[57] There is a potential large prediction error on the individual level with BIA. Indeed, there is a systematic positive bias with an overall underestimation of lean body mass measurements by BIA.[60] It is, however, one of the few alternatives when other more precise techniques are not feasible.

Emerging techniques for the assessment of muscle mass

Because of the limitations of the current techniques to assess lean mass (cost, accuracy, feasibility), new techniques have

Table 2 Strengths and weakness of estimating muscle mass by BIA

Strengths	Weaknesses
Inexpensive and easy to use[4]	Measurements are sensitive to subjects' conditions such as hydration, recent activity, and time being horizontal[58,59]
Precise measurement of body resistance and reactance	Large individual prediction error for estimated muscle mass
Safe and non-invasive method[17]	Need of age, gender, and ethnic-specific prediction equation to estimate muscle mass
Portable tool and can be used in most environments[57]	No BIA-specific equations validated in patients with extreme BMI
Does not require highly trained personnel	Multiple devices with different body composition outputs

BIA, bioelectrical impedance analysis; BMI, body mass index.

appeared. Among these techniques, creatine (*methyl*-d3) dilution (D3-creatine) is of some interest.[61]

Creatine is present predominantly (~95%) in skeletal muscle. Roughly 2% of creatine is converted to creatinine per day, via an irreversible, non-enzymatic mechanism, so that ~2 g per day of creatine are replaced in the whole body. Based on the assumption that conversion of creatine to creatinine is constant among and within subjects, the daily excretion rate of creatinine has been used as a metric of whole body creatine pool size.[62] Reviews of this method show that a relatively broad range of muscle mass per gram of urinary creatinine (17–22 kg) has been used to estimate muscle mass, leading to large variability in muscle mass estimates between studies, and further suggest limitations to this method in certain patient groups, such as those affected by renal failure.[63] Furthermore, there are inherent limitations to this method (in addition to the problem of inaccurate 24-h urine collections): pH and temperature affect the non-enzymatic conversion rate of creatine to creatinine, and there is degradation and metabolic removal of creatinine in the body, so all creatinine produced is not excreted in the urine.[64] The results are also dependent on the intake of meat that increases the excretion of creatinine. Thus, accurate assessment requires a meat-free diet for about 1–2 weeks.

Electrical impedance myography is a non-invasive, painless approach to muscle assessment based on the application and measurement of high-frequency, low-intensity electrical current. Measurements are made over a small area of interest, with energy being applied to the body and the resultant surface patterns analysed. Several parameters are obtained, including the tissue's reactance, resistance, and phase angle that can provide a quantitative measure of muscle condition.[65] The central concept of electrical impedance myography is that skeletal muscle can be modelled as a network of resistors and capacitors. The intracellular and extracellular matrices of muscle tissue act as resistors, and any atrophy that reduces the cross-sectional area of muscle tissue would be expected to increase the resistance. The lipid bilayers that constitute muscle membranes act as capacitors, and as muscle atrophies, the cumulative capacitance of the muscle membranes increases.[66] Electrical current is used, and the output is a set of quantitative parameters describing muscle state, with presently little emphasis on imaging (though this remains possible).[67]

Ultrasound is an imaging technique that can determine thickness and cross-sectional areas of superficial muscles. In particular, with ultrasound analysis, it is possible to measure key parameters of muscle architecture, such as muscle volume, fascicle length, and pennation angle. Fascicle length, which is an estimate of muscle fibre length, is defined as the length of a line coincident with the fascicle between the deep and superficial aponeuroses. Fascicle length indicates the range of lengths over which the muscle is capable of actively producing force, known as the excursion potential. Pennation angle represents the angle of the muscle fibres that constitute a muscle fascicle relative to the force-generating axis, and directly affects both the force production and the excursion; larger angles of pennation limiting the excursion potential.[68] Ultrasound has the advantage of being portable and involves no ionizing radiation. A number of studies have confirmed the reliability of this technique for measuring the size of the quadriceps muscle in health. For example, an ICC of 0.97 (95%CI: 0.92–0.99) was found for the test–retest reliability of ultrasound at the rectus femoris.[69] However, a major problem is the impact of the applied pressure on the probe on the measurement result. Even though, this method of body composition analysis is not widely used for sarcopenia screening and staging,[70,71] in the near future, it may become a valid method to assess muscle in different settings.[72]

Biomarkers are another way to assess muscle mass. Previous studies have shown that the serum levels of the Collagen type III propeptide correlate well with whole body lean mass.[73] As do the circulating levels of Collagen type VI peptides containing the IC6 epitope.[74] Nedergaard et al. shown that the anabolic response to reloading the following immobilization was inversely related to the levels of the matrix-metalloproteinase-generated Collagen type VI fragment C6M.[74]Both Collagen types III and VI are known to be important constituents of the extracellular matrix of skeletal muscle.[75,76] Therefore, fragments produced during muscle tissue turnover may be correlated with lean body mass.[73] Dysregulation of microRNAs may also contribute to reduced muscle plasticity with aging.[77]

Towards a reference standard

The considerations above indicate that no currently available technique serves all the requirements for the measurement of muscle mass. Each has limitations and in particular, there is a dearth of information on accuracy. Moreover, none are fully standardized. Thus, there is at present no gold standard. Notwithstanding, there is need to develop a reference standard against which alternative techniques can be evaluated.

Major disadvantages of CT are limited access to the radiological departments that operate it, considerably higher cost and radiation exposure than for DXA. Limitations in the use of MRI in clinical and research settings are largely related to the high cost and the technical expertise required for analysis and limited access. The main challenge for BIA is the availability of population-specific equations to predict lean mass (or other body composition parameters) according to the reference standard used to validate the BIA equation. In fact, several good equations are available; but many clinicians rely on the outputs generated by the device itself (which is using an in-built equation, most often kept 'hidden' by the manufacturer).

These considerations suggest that, despite many limitations,[78] DXA may be considered the current reference technique for assessing muscle mass and body composition in research and clinical practice. An important reason for preferring DXA above BIA is that DXA measures body composition on an individual level, whilst BIA uses a prediction equation (so it estimates muscle instead of measuring it), and is hampered by large prediction error on the individual level. Also, BIA standardization will be more complicated than DXA standardization due to the multitude of available BIA devices.

In addition, DXA has been used successfully to estimate skeletal muscle mass as part of RCT's.[79–81] Currently, it is the preferred and effective measurement technique in this context.

To ensure the accuracy of DXA measurement, standardization is needed. Calibration materials and equations used to derive lean mass should be standardized across manufacturers. An important item on the research agenda is to standardize the local regions of Interest, such as trunk, arms, legs, that are significantly different across manufacturers. Finally, consensus is required in adopting a reference population in much the same way as has been achieved for the use of DXA in osteoporosis.[82]

It is important to note that the adoption of a reference standard does not proscribe the use of any of the techniques in clinical research or clinical practice. Indeed, this is to be encouraged. There is a useful analogy with the use of BMD in the assessment of osteoporosis. The reference standard is BMD at the femoral neck,[83] but in clinical research and clinical practice many assessment tools are widely used (e.g. BMD at other skeletal sites, CT, quantitative ultrasound, and trabecular bone score). The caveat is that where the opportunity arises BMD should also be reported using the reference technology applied to a reference population and is now a requirement in many of the bone journals.

The adoption of DXA as a reference standard with a defined normal range provides a platform on which the performance characteristics of less well-established and new methodologies can be compared. It also permits comparisons between studies and between countries.

Different indices to express lean body mass

Skeletal muscle index (SMI) is a measure to express lean mass in relation to height or weight. Unfortunately, the common use and terminology of SMI is inconsistent.[84] It is either defined as appendicular skeletal muscle mass divided by height^2 and measured in kg/m^2[22] or as skeletal muscle mass divided by body mass × 100, which is a unitless index,[24] although some authors distinguish them as appendicular lean mass/ht^2 and SMI.[85] SMI can be derived from BIA or from DXA measurements. Both SMI definitions have previously been shown to predict disability and functional limitations in large, epidemiologic studies of older adults.[8,86,87] However, the classification of community-dwelling older adults as sarcopenic or non-sarcopenic differed markedly for the two definitions.[85] The weight based SMI classified significantly more community-dwelling older adults as sarcopenic than the height based index, a trend more deep-seated in men than women. More recently even a third definition, the application lifecycle management/BMI index was proposed.[8,85] Due to the discrepancies observed, a clearer terminology should be developed, and it seems necessary to use that index that can best describe the associations between muscle mass and important clinical outcomes in epidemiological studies.

Discussion

More and more attention is being paid to the measurement of muscle mass in different contexts and populations. Over the last few years, sarcopenia has come into the spotlight in biogerontology and research. In this context, the establishment of an international consensus on an accurate, reliable, and cost-effective method to assess muscle mass across research and clinical settings is of utmost importance. As shown above, a wide range of techniques can be used to estimate or measure muscle mass.[9] Cost, availability, and ease of use can determine whether the techniques are better suited to clinical practice or are more useful for research. Reference standards are well established in the pharmaceutical industry and laboratory settings, and refer to 'a universal reference method that performs equally and reproducibly between platforms'. In the field of musculoskeletal disorder, there is no reference method for measuring muscle mass, and this paper aims to begin filling this gap.

A muscle mass measure should provide a diagnostic criterion for sarcopenia, prognostic information, and a baseline to monitor the natural history of treated and untreated patients. To obtain a complete picture of body composition, a 4-component model comprising total body water, protein, mineral, and fat mass is required. However, this is a highly intensive and costly procedure and does not enable the measurement of muscle mass specifically.[88] As a 3-component model (combining protein and minerals into 'solids'), DXA is superior to standard densitometry (which differentiates only between fat mass and fat-free mass), and has been widely adopted.[9] DXA is primarily used for diagnosing osteoporosis, assessing fracture risk, and

DOI: 10.1002/jcsm.12268

monitoring therapy. However, given its ability to measure soft tissue mass of the arms and legs as an accurate and precise assessment of appendicular skeletal muscle mass, DXA has been suggested as a potential tool for diagnosing sarcopenia.[4] Operationally, DXA-based definitions for sarcopenia have generally used appendicular skeletal muscle mass divided by body height squared.[89] However, more recently, the ratio of appendicular skeletal muscle mass divided by BMI is also being suggested.[8] Regardless of the choice of outcome measure for clinical trials of sarcopenia, precise methodology is available for the assessment of lean mass, and this may serve as a key defining characteristic of sarcopenia in clinical practice.[9]

DXA has now largely met that unmet need by providing a measure of muscle mass at relatively low cost and with minimal radiation exposure.[32] Thus, it could be considered as the reference standard for measuring muscle mass and for providing a platform on which the performance characteristics of less well-established and new methodologies can be compared.

We conclude that the adoption of reference standards will contribute to the development of the assessment of muscle mass, in order to make studies comparable and to improve the diagnosis and treatment of sarcopenia, but also to monitor the development of muscle mass in healthy, athletic, and sick subjects. In this sense, DXA provides a precise measure of lean mass, but further standardization is needed to ensure that the assessment and cut points are used accurately on all makes and models of DXA systems. Thus, the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis working group on frailty and sarcopenia state that DXA is the gold standard for the measurement of muscle mass.

Acknowledgements

The authors certify that they comply with the ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2015.[90]

The working group and this paper were fully funded by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO), a Belgian not-for-profit organization. ESCEO was responsible for the selection of participants to the preliminary meeting and for the choice of the authors of the manuscript, covering all expenses related to the organization of the preliminary meeting and the presentation of outcomes of the working group at the 2017 World Congress on Osteoporosis, Osteoarthritis, and Musculoskeletal Diseases (in Florence). ESCEO also covered all expenses pertaining to the preparation, writing, and submission of the manuscript.

We acknowledge the valuable input of J. Beard, J. Amuthavalli Thiyagarajan, and I. Araujo de Carvalho (World Health Organization, Geneva, Switzerland). R.A.F.'s participation was partially supported by the U.S. Department of Agriculture – Agricultural Research Service (ARS), under Agreement No. 58–1950–4-003. The views expressed do not reflect those of the United States Department of Agriculture.

Conflict of Interest

F.B., F.L., M.C., M.V., K.E., S.M., E.D., N.A.-D., S.A., J.B., I.B., M.-L.B., O.B., T.C., F.C., A.C., C.C., A.C.-J., E.M., B.D.-H., J.-M. K., A.L., J.P., J.-Y.R., R.R., S.R., Y.R., R.R., B.V., and J.A.K. declare that they have no conflict of interest.

References

1. Rosenberg IH. Sarcopenia: origins and clinical relevance. *J Nutr* 1997;**127**:990s–991s.
2. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ, Lindeman RD. Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol* 1998;**147**:755–763.
3. Muscaritoli M, Anker SD, Argiles J, Aversa Z, Bauer JM, Biolo G, Boirie Y, Bosaeus I, Cederholm T, Costelli P, et al. Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics". *Clin Nutr* 2010;**29**:154–159.
4. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* 2010;**39**:412–423.
5. Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, Newman AB. Abellan van Kan G, Andrieu S, Bauer J, Breuille D, et al: Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. *J Am Med Dir Assoc* 2011;**12**:249–256.
6. Dam TT, Peters KW, Fragala M, Cawthon PM, Harris TB, McLean R, Shardell M, Alley DE, Kenny A, Ferrucci L, et al. An evidence-based comparison of operational criteria for the presence of sarcopenia. *J Gerontol A Biol Sci Med Sci* 2014;**69**:584–590.
7. Morley JE, Abbatecola AM, Argiles JM, Baracos V, Bauer J, Bhasin S, Cederholm T, Coats AJ, Cummings SR, Evans WJ, et al. Sarcopenia with limited mobility: an international consensus. *J Am Med Dir Assoc* 2011;**12**:403–409.
8. Studenski SA, Peters KW, Alley DE, Cawthon PM, McLean RR, Harris TB, Ferrucci L, Guralnik JM, Fragala MS, Kenny AM, et al. The FNIH sarcopenia project: rationale, study description, conference recommendations, and final estimates. *J Gerontol A Biol Sci Med Sci* 2014;**69**:547–558.
9. Cooper C, Dere W, Evans W, Kanis JA, Rizzoli R, Sayer AA, Sieber CC, Kaufman JM. Abellan van Kan G, Boonen S, et al: Frailty and sarcopenia: definitions and outcome parameters. *Osteoporos Int* 2012;**23**:1839–1848.
10. Chen LK, Liu LK, Woo J, Assantachai P, Auyeung TW, Bahyah KS, Chou MY, Chen LY, Hsu PS, Krairit O, et al. Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. *J Am Med Dir Assoc* 2014;**15**:95–101.

11. Marzetti E. Editorial: imaging, functional and biological markers for sarcopenia: the pursuit of the golden ratio. *J Frailty Aging* 2012;**1**:97–98.
12. Beaudart C, McCloskey E, Bruyere O, Cesari M, Rolland Y, Rizzoli R. Araujo de Carvalho I, Amuthavalli Thiyagarajan J, Bautmans I, Bertiere MC, et al: Sarcopenia in daily practice: assessment and management. *BMC Geriatr* 2016;**16**:170.
13. Cruz-Jentoft AJ, Landi F. Sarcopenia. *Clin Med (Lond)* 2014;**14**:183–186.
14. Cesari M, Fielding RA, Pahor M, Goodpaster B, Hellerstein M, van Kan GA, Anker SD, Rutkove S, Vrijbloed JW, Isaac M, et al. Biomarkers of sarcopenia in clinical trials-recommendations from the International Working Group on Sarcopenia. *J Cachexia Sarcopenia Muscle* 2012;**3**:181–190.
15. Lustgarten MS, Fielding RA. Assessment of analytical methods used to measure changes in body composition in the elderly and recommendations for their use in phase II clinical trials. *J Nutr Health Aging* 2011;**15**:368–375.
16. Mijnarends DM, Meijers JM, Halfens RJ, ter Borg S, Luiking YC, Verlaan S, Schoberer D, Cruz Jentoft AJ, van Loon LJ, Schols JM. Validity and reliability of tools to measure muscle mass, strength, and physical performance in community-dwelling older people: a systematic review. *J Am Med Dir Assoc* 2013;**14**:170–178.
17. Heymsfield SB, Gonzalez MC, Lu J, Jia G, Zheng J. Skeletal muscle mass and quality: evolution of modern measurement concepts in the context of sarcopenia. *Proc Nutr Soc* 2015;**74**:355–366.
18. Kuriyan R, Thomas T, Ashok S, Jayakumar J, Kurpad AV. A 4-compartment model based validation of air displacement plethysmography, dual energy X-ray absorptiometry, skinfold technique & bio-electrical impedance for measuring body fat in Indian adults. *Indian J Med Res* 2014;**139**:700–707.
19. Dawson-Hughes B, Bischoff-Ferrari H. Considerations concerning the definition of sarcopenia. *Osteoporos Int* 2016;**27**:3139–3144.
20. Matiegka J. The testing of physical efficiency. *Am J Phys Anthropol* 1921;**4**:223–230.
21. Hounsfield GN. Computerized transverse axial scanning (tomography): Part I. Description of system. 1973. *Br J Radiol* 1995;**68**:H166–H172.
22. Bruyere OB, Beaudart C, Reginster J-Y, Buckinx F, Schoene D, Hirani V, Cooper C, Kanis J-A, Rizzoli R, McCloskey E, Cederholm T, Cruz-Jentoft A, Freiberger E. Assessment of muscle mass, muscle strength and physical performance in clinical practice: an international survey. *European Geriatric Medicine* 2016;https://doi.org/10.1016/j.eurger.2015.12.009.
23. Scafoglieri A, Deklerck R, Tresignie J, De Mey J, Clarys JP, Bautmans I. Assessment of regional adipose tissue depots: a DXA and CT comparison in cadavers of elderly persons. *Exp Gerontol* 2013;**48**:985–991.
24. Janssen I, Heymsfield SB, Wang ZM, Ross R. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *J Appl Physiol (1985)* 2000;**89**:81–88.
25. Kim J, Wang Z, Heymsfield SB, Baumgartner RN, Gallagher D. Total-body skeletal muscle mass: estimation by a new dual-energy X-ray absorptiometry method. *Am J Clin Nutr* 2002;**76**:378–383.
26. Dittmar M, Reber H. New equations for estimating body cell mass from bioimpedance parallel models in healthy older Germans. *Am J Physiol Endocrinol Metab* 2001;**281**:E1005–E1014.
27. Erlandson MC, Lorbergs AL, Mathur S, Cheung AM. Muscle analysis using pQCT, DXA and MRI. *Eur J Radiol* 2016;**85**:1505–1511.
28. Blake GM, Fogelman I. Technical principles of dual energy x-ray absorptiometry. *Semin Nucl Med* 1997;**27**:210–228.
29. Quantitative aspects of bone densitometry: contents**.** *J icru* 2009, **9:**Np.
30. Pietrobelli A, Formica C, Wang Z, Heymsfield SB. Dual-energy X-ray absorptiometry body composition model: review of physical concepts. *Am J Physiol* 1996;**271**: E941–E951.
31. Maden-Wilkinson TM, Degens H, Jones DA, McPhee JS. Comparison of MRI and DXA to measure muscle size and age-related atrophy in thigh muscles. *J Musculoskelet Neuronal Interact* 2013;**13**:320–328.
32. Heymsfield SB, Adamek M, Gonzalez MC, Jia G, Thomas DM. Assessing skeletal muscle mass: historical overview and state of the art. *J Cachexia Sarcopenia Muscle* 2014;**5**:9–18.
33. Visser M, Fuerst T, Lang T, Salamone L, Harris TB. Validity of fan-beam dual-energy X-ray absorptiometry for measuring fat-free mass and leg muscle mass. Health, aging, and body composition study – dual-energy X-ray absorptiometry and body composition working group. *J Appl Physiol (1985)* 1999;**87**:1513–1520.
34. Bredella MA, Ghomi RH, Thomas BJ, Torriani M, Brick DJ, Gerweck AV, Misra M, Klibanski A, Miller KK. Comparison of DXA and CT in the assessment of body composition in premenopausal women with obesity and anorexia nervosa. *Obesity (Silver Spring)* 2010;**18**:2227–2233.
35. Bilsborough JC, Greenway K, Opar D, Livingstone S, Cordy J, Coutts AJ. The accuracy and precision of DXA for assessing body composition in team sport athletes. *J Sports Sci* 2014;**32**:1821–1828.
36. Carver TE, Christou NV, Andersen RE. In vivo precision of the GE iDXA for the assessment of total body composition and fat distribution in severely obese patients. *Obesity (Silver Spring)* 2013;**21**:1367–1369.
37. Hind K, Oldroyd B. In-vivo precision of the GE Lunar iDXA densitometer for the measurement of appendicular and trunk lean and fat mass. *Eur J Clin Nutr* 2013;**67**:1331–1333.
38. Knapp KM, Welsman JR, Hopkins SJ, Shallcross A, Fogelman I, Blake GM. Obesity increases precision errors in total body dual-energy x-ray absorptiometry measurements. *J Clin Densitom* 2015;**18**:209–216.
39. Toombs RJ, Ducher G, Shepherd JA, De Souza MJ. The impact of recent technological advances on the trueness and precision of DXA to assess body composition. *Obesity (Silver Spring)* 2012;**20**:30–39.
40. Hangartner TN, Warner S, Braillon P, Jankowski L, Shepherd J. The official positions of the international society for clinical densitometry: acquisition of dual-energy X-ray absorptiometry body composition and considerations regarding analysis and repeatability of measures. *J Clin Densitom* 2013;**16**:520–536.
41. Damilakis J, Adams JE, Guglielmi G, Link TM. Radiation exposure in X-ray-based imaging techniques used in osteoporosis. *Eur Radiol* 2010;**20**:2707–2714.
42. Prado CM, Heymsfield SB. Lean tissue imaging: a new era for nutritional assessment and intervention. *JPEN J Parenter Enteral Nutr* 2014;**38**:940–953.
43. Rothney MP, Brychta RJ, Schaefer EV, Chen KY, Skarulis MC. Body composition measured by dual-energy X-ray absorptiometry half-body scans in obese adults. *Obesity (Silver Spring)* 2009;**17**:1281–1286.
44. Genant HK, Grampp S, Gluer CC, Faulkner KG, Jergas M, Engelke K, Hagiwara S, Van Kuijk C. Universal standardization for dual x-ray absorptiometry: patient and phantom cross-calibration results. *J Bone Miner Res* 1994;**9**:1503–1514.
45. Hull H, He Q, Thornton J, Javed F, Allen L, Wang J, Pierson RN Jr, Gallagher D. iDXA, prodigy, and DPXL dual-energy X-ray absorptiometry whole-body scans: a cross-calibration study. *J Clin Densitom* 2009;**12**:95–102.
46. Saarelainen J, Hakulinen M, Rikkonen T, Kroger H, Tuppurainen M, Koivumaa-Honkanen H, Honkanen R, Hujo M, Jurvelin JS. Cross-calibration of GE healthcare lunar prodigy and iDXA dual-energy X-ray densitometers for bone mineral measurements. *J Osteoporos* 2016;**2016**: 1424582.
47. Snyder WSC, Cook MJ, Nasset ES, Karhansen LR, Howells GP, Tipton IH. *Report of the task group on reference men*. Oxford, United Kingdom: Pergamon Press; 1975.
48. Daguet E, Jolivet E, Bousson V, Boutron C, Dahmen N, Bergot C, Vicaut E, Laredo JD. Fat content of hip muscles: an anteroposterior gradient. *J Bone Joint Surg Am* 2011;**93**:1897–1905.
49. Paulus MJ, Gleason SS, Kennel SJ, Hunsicker PR, Johnson DK. High resolution X-ray computed tomography: an emerging tool for small animal cancer research. *Neoplasia* 2000;**2**:62–70.
50. Strandberg S, Wretling ML, Wredmark T, Shalabi A. Reliability of computed tomography measurements in assessment of thigh muscle cross-sectional area and attenuation. *BMC Med Imaging* 2010;**10**:18.
51. Heymsfield SB, Wang Z, Baumgartner RN, Ross R. Human body composition: advances in models and methods. *Annu Rev Nutr* 1997;**17**:527–558.

52. Ross R, Rissanen J, Pedwell H, Clifford J, Shragge P. Influence of diet and exercise on skeletal muscle and visceral adipose tissue in men. *J Appl Physiol (1985)* 1996;**81**:2445–2455.
53. Hoffer EC, Meador CK, Simpson DC. Correlation of whole-body impedance with total body water volume. *J Appl Physiol* 1969;**27**:531–534.
54. Nyboer J. Workable volume and flow concepts of bio-segments by electrical impedance plethysmography. 1972. *Nutrition* 1991;**7**:396–408, discussion 409.
55. Thomasset A. Bioelectrical properties of tissue impedance measurements. *Lyon Med* 1962;**94**:107–118.
56. Khalil SF, Mohktar MS, Ibrahim F. The theory and fundamentals of bioimpedance analysis in clinical status monitoring and diagnosis of diseases. *Sensors (Basel)* 2014;**14**:10895–10928.
57. Buckinx F, Reginster JY, Dardenne N, Croisiser JL, Kaux JF, Beaudart C, Slomian J, Bruyere O. Concordance between muscle mass assessed by bioelectrical impedance analysis and by dual energy X-ray absorptiometry: a cross-sectional study. *BMC Musculoskelet Disord* 2015;**16**:60.
58. Bioelectrical impedance analysis in body composition measurement: national institutes of health technology assessment conference statement. *Am J Clin Nutr* 1996;**64**:524s–532s.
59. Sergi G, Coin A, Marin S, Vianello A, Manzan A, Peruzza S, Inelmen EM, Busetto L, Mulone S, Enzi G. Body composition and resting energy expenditure in elderly male patients with chronic obstructive pulmonary disease. *Respir Med* 2006;**100**:1918–1924.
60. Ling CH, de Craen AJ, Slagboom PE, Gunn DA, Stokkel MP, Westendorp RG, Maier AB. Accuracy of direct segmental multi-frequency bioimpedance analysis in the assessment of total body and segmental body composition in middle-aged adult population. *Clin Nutr* 2011;**30**:610–615.
61. Clark RV, Walker AC, O'Connor-Semmes RL, Leonard MS, Miller RR, Stimpson SA, Turner SM, Ravussin E, Cefalu WT, Hellerstein MK, Evans WJ. Total body skeletal muscle mass: estimation by creatine (methyl-d3) dilution in humans. *J Appl Physiol (1985)* 2014;**116**:1605–1613.
62. Crim MC, Calloway DH, Margen S. Creatine metabolism in men: urinary creatine and creatinine excretions with creatine feeding. *J Nutr* 1975;**105**:428–438.
63. Heymsfield SB, Arteaga C, McManus C, Smith J, Moffitt S. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am J Clin Nutr* 1983;**37**:478–494.
64. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. *Physiol Rev* 2000;**80**:1107–1213.
65. Li J, Spieker AJ, Rosen GD, Rutkove SB. Electrical impedance alterations in the rat hind limb with unloading. *J Musculoskelet Neuronal Interact* 2013;**13**:37–44.
66. Tarulli AW, Duggal N, Esper GJ, Garmirian LP, Fogerson PM, Lin CH, Rutkove SB. Electrical impedance myography in the assessment of disuse atrophy. *Arch Phys Med Rehabil* 2009;**90**:1806–1810.
67. Rutkove SB. Electrical impedance myography: background, current state, and future directions. *Muscle Nerve* 2009;**40**:936–946.
68. Stevens DE, Smith CB, Harwood B, Rice CL. In vivo measurement of fascicle length and pennation of the human anconeus muscle at several elbow joint angles. *J Anat* 2014;**225**:502–509.
69. Thomaes T, Thomis M, Onkelinx S, Coudyzer W, Cornelissen V, Vanhees L. Reliability and validity of the ultrasound technique to measure the rectus femoris muscle diameter in older CAD-patients. *BMC Med Imaging* 2012;**12**:7.
70. Ismail C, Zabal J, Hernandez HJ, Woletz P, Manning H, Teixeira C, DiPietro L, Blackman MR, Harris-Love MO. Diagnostic ultrasound estimates of muscle mass and muscle quality discriminate between women with and without sarcopenia. *Front Physiol* 2015;**6**:302.
71. Menon MK, Houchen L, Harrison S, Singh SJ, Morgan MD, Steiner MC. Ultrasound assessment of lower limb muscle mass in response to resistance training in COPD. *Respir Res* 2012;**13**:119.
72. Mueller N, Murthy S, Tainter CR, Lee J, Riddell K, Fintelmann FJ, Grabitz SD, Timm FP, Levi B, Kurth T, Eikermann M. Can sarcopenia quantified by ultrasound of the rectus femoris muscle predict adverse outcome of surgical intensive care unit patients as well as frailty? A prospective, observational cohort study. *Ann Surg* 2016;**264**:1116–1124.
73. Nedergaard A, Dalgas U, Primdahl H, Johansen J, Overgaard J, Overgaard K, Henriksen K, Karsdal MA, Lonbro S. Collagen fragment biomarkers as serological biomarkers of lean body mass – a biomarker pilot study from the DAHANCA25B cohort and matched controls. *J Cachexia Sarcopenia Muscle* 2015;**6**:335–342.
74. Nedergaard A, Sun S, Karsdal MA, Henriksen K, Kjaer M, Lou Y, He Y, Zheng Q, Suetta C. Type VI collagen turnover-related peptides-novel serological biomarkers of muscle mass and anabolic response to loading in young men. *J Cachexia Sarcopenia Muscle* 2013;**4**:267–275.
75. Urciuolo A, Quarta M, Morbidoni V, Gattazzo F, Molon S, Grumati P, Montemurro F, Tedesco FS, Blaauw B, Cossu G, et al. Collagen VI regulates satellite cell self-renewal and muscle regeneration. *Nat Commun* 2013;**4**:1964.
76. Sabatelli P, Gualandi F, Gara SK, Grumati P, Zamparelli A, Martoni E, Pellegrini C, Merlini L, Ferlini A, Bonaldo P, et al. Expression of collagen VI alpha5 and alpha6 chains in human muscle and in Duchenne muscular dystrophy-related muscle fibrosis. *Matrix Biol* 2012;**31**:187–196.
77. Rivas DA, Lessard SJ, Rice NP, Lustgarten MS, So K, Goodyear LJ, Parnell LD, Fielding RA. Diminished skeletal muscle microRNA expression with aging is associated with attenuated muscle plasticity and inhibition of IGF-1 signaling. *FASEB J* 2014;**28**:4133–4147.
78. Roubenoff R, Kehayias JJ, Dawson-Hughes B, Heymsfield SB. Use of dual-energy x-ray absorptiometry in body-composition studies: not yet a "gold standard". *Am J Clin Nutr* 1993;**58**:589–591.
79. Stewart Coats AJ, Ho GF, Prabhash K, von Haehling S, Tilson J, Brown R, Beadle J, Anker SD. Espindolol for the treatment and prevention of cachexia in patients with stage III/IV non-small cell lung cancer or colorectal cancer: a randomized, double-blind, placebo-controlled, international multicentre phase II study (the ACT-ONE trial). *J Cachexia Sarcopenia Muscle* 2016;**7**:355–365.
80. van de Bool C, Rutten EPA, van Helvoort A, Franssen FME, Wouters EFM, Schols A. A randomized clinical trial investigating the efficacy of targeted nutrition as adjunct to exercise training in COPD. *J Cachexia Sarcopenia Muscle* 2017;**8**:748–758.
81. Temel JS, Abernethy AP, Currow DC, Friend J, Duus EM, Yan Y, Fearon KC. Anamorelin in patients with non-small-cell lung cancer and cachexia (ROMANA 1 and ROMANA 2): results from two randomised, double-blind, phase 3 trials. *Lancet Oncol* 2016;**17**:519–531.
82. Technical standardization for dual-energy x-ray absorptiometry. *J Clin Densitom* 2004;**7**:27–36.
83. Kanis JA, Adachi JD, Cooper C, Clark P, Cummings SR, Diaz-Curiel M, Harvey N, Hiligsmann M, Papaioannou A, Pierroz DD, et al. Standardising the descriptive epidemiology of osteoporosis: recommendations from the Epidemiology and Quality of Life Working Group of IOF. *Osteoporos Int* 2013;**24**:2763–2764.
84. Kim KM, Jang HC, Lim S. Differences among skeletal muscle mass indices derived from height-, weight-, and body mass index-adjusted models in assessing sarcopenia. *Korean J Intern Med* 2016;**31**:643–650.
85. Merriwether EN, Host HH, Sinacore DR. Sarcopenic indices in community-dwelling older adults. *J Geriatr Phys Ther* 2012;**35**:118–125.
86. Janssen I, Baumgartner RN, Ross R, Rosenberg IH, Roubenoff R. Skeletal muscle cutpoints associated with elevated physical disability risk in older men and women. *Am J Epidemiol* 2004;**159**:413–421.
87. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc* 2002;**50**:889–896.
88. Plank LD. Dual-energy X-ray absorptiometry and body composition. *Curr Opin Clin Nutr Metab Care* 2005;**8**:305–309.
89. Sirola J, Kroger H. Similarities in acquired factors related to postmenopausal osteoporosis and sarcopenia. *J Osteoporos* 2011;**2011**:536735.
90. von Haehling S, Morley JE, Coats AJ, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2015. *J Cachexia Sarcopenia Muscle* 2015;**6**:315–316.